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Translocation of Photosynthetic Carbon From Two Algal Symbionts to the Sea Anemone *Anthopleura elegantissima*

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Abstract. The intertidal sea anemone *Anthopleura elegantissima* contains two symbiotic algae, zoochlorellae and zooxanthellae, in the Northern Puget Sound region. Possible nutritional advantages to hosting one algal symbiont over the other were explored by comparing the photosynthetic and carbon translocation rates of both symbionts under different environmental conditions. Each alga translocated 30% of photosynthetically fixed carbon in freshly collected anemones, although zoochlorellae fixed and translocated less carbon than zooxanthellae. The total amount of carbon translocated to the host was equivalent because densities of zoochlorellae were two to three times greater than were densities of zooxanthellae. In *A. elegantissima* maintained under high and low irradiance (100 and 10 μmol photons/m^2/s) at 20°C and 13°C for 21 days, both algae fixed and translocated carbon at greater rates at 20°C (translocation rates: 0.38 pg C /zoochlorella/h; 1.12 pg C /zooxanthella/h) than at 13°C (translocation rates: 0.06 pg C /zoochlorella/h; 0.37 pg C /zooxanthella/h). However, zoochlorellate anemones received 3.5 times less carbon at 20°C than at 13°C because the higher temperature caused a significant reduction in the density of zoochlorellae. Environmental variables, like temperature, that influence the densities of the two symbionts will affect their relative nutritional contribution to the host. Whether these differences in carbon translocation rates of the two algal symbionts affect the ecology of their anemone host awaits further investigation.

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Introduction

The temperate sea anemones *Anthopleura elegantissima* and *Anthopleura xanthogrammica* host both dinoflagellate zooxanthellae and green algae known only generally as zoochlorellae (Muscatine, 1971). Both algal symbionts photosynthetically fix inorganic carbon and translocate some of the products to the animal host. Zooxanthellae in corals, as well as in *A. elegantissima*, translocate carbon to the host mainly as glycerol (Muscatine, 1967; Trench, 1971; Battey and Patton, 1987). Glycerol is used by the host to support its basal metabolism, while lipids that are also translocated by the algae are used to create lipid stores (Battey and Patton, 1987). We do not know what products are translocated by marine zoochlorellae to their host, although unpublished work by Minnick and McCloskey (cited in Verde and McCloskey, 1996) indicates that zoochlorellae translocate several amino acids in addition to glycerol. For zoochlorellae in the freshwater green hydra, maltose is the principal form of translocated photosynthate (Mews and Smith, 1982).

Further understanding of the nutritional relationship between *Anthopleura* and the two algae may come from comparisons of the amount of carbon translocated from the algae to the host. Previous studies have suggested that zoochlorellae do not translocate as much carbon as zooxanthellae. Using ^{14}C, O'Brien (1980) found that zoochlorellae in excised tentacles translocate from zero to 3.6% of the total carbon fixed by the algae to the epidermal tissues of *Anthopleura xanthogrammica*. Zooxanthellae in intact anemones translocate as much as 50% of the total ^{14}C-labelled carbon fixed to the host fraction of *A. elegantissima* (Trench, 1971). Based on carbon budgets, Verde and McCloskey (1996) calculate that zooxanthellae will have photosynthetic products available to supply *A. elegantissima*

with 48% of its respiratory carbon requirement, while zoochlorellae will only be able to satisfy 9% of the anemone's respiratory needs. Verde and McCloskey conclude that the higher net photosynthesis and lower algal growth demand of zooxanthellae combine to provide more photosynthetic carbon to a zooxanthellate host anemone than is the case for an anemone that contains zoochlorellae as its endosymbiont. These studies show that zooxanthellae appear to be the "better" symbiont with respect to carbon supplied to the host.

It is important to directly compare carbon translocation rates of zoochlorellae and zooxanthellae under different temperatures and irradiance levels, because intertidal *A. elegantissima* are exposed to extreme seasonal fluctuations in these parameters (Dingman, 1998). Furthermore, both irradiance and temperature are thought to influence the distribution of these two algae within anemones. Field observations of the distribution of *Anthopleura xanthogrammica* in British Columbia, Canada, by O'Brien and Wyttenbach (1980) led the authors to suggest that zooxanthellae and zoochlorellae populations in anemones may be regulated by temperature. In the lower latitude, warmer regions of *Anthopleura's* range zooxanthellae are the dominant symbiont, while zoochlorellae are more abundant in anemones in the higher latitude, colder regions of *Anthopleura's* range (Secord, 1995). Are these distribution patterns related to differences in carbon translocation of the two algae? Saunders and Muller-Parker (1997) determined that increased temperature caused a reduction in the density of zoochlorellae in *Anthopleura elegantissima* tentacles over time. How do such changes in algal density affect the rate of carbon translocation to the host?

This study compares carbon fixation and translocation rates of both zoochlorellate and zooxanthellate anemones collected from a single site and kept under different environmental conditions likely to be encountered in the field. The effects of irradiance and temperature on translocation of fixed carbon from zooxanthellae and zoochlorellae to *A. elegantissima* are examined by measuring the distribution of radioactively labelled carbon in the algae and in the animal host, and relating the carbon translocation rates to population densities of the respective algae.

Materials and Methods

Collection of anemones and determination of symbiont complement

Anthopleura elegantissima was collected from a rocky intertidal area located on Anaco Beach, Fidalgo Island, Washington (48° 29′; 122° 42′) in June and July of 1994. Ambient seawater temperature was 11°C. Both zooxanthellate and zoochlorellate anemones were collected from the same large boulder, at one tidal height (+0.6 m). Nonsymbiotic (algae-free) anemones were collected from dark crevices in a nearby rock jetty. The anemones were placed in flow-through ambient seawater tables at Shannon Point Marine Center for one day before experiments began.

The anemones were separated by color and excised tentacles from several anemones were examined microscopically to verify that anemones that appeared brown in the field actually contained zooxanthellae, that green anemones contained zoochlorellae, and that white anemones were algae-free.

The symbiont complement of all anemones was confirmed by counting the number of zoochlorellae and zooxanthellae in homogenized anemone samples after ^{14}C incubation. Zoochlorellate anemones from the field contained an average of 99.0% (±2.0 SD, $n = 18$) zoochlorellae, while zooxanthellate anemones contained an average of 97.3% (±3.2 SD, $n = 18$) zooxanthellae. Three field anemones that contained mixed populations of both symbionts contained from 40% to 60% of each alga (average = 53% zoochlorellae) within their tissues.

Experimental treatments: symbiont, light, and temperature

To examine the effects of irradiance and temperature on zooxanthellate and zoochlorellate anemones, a 2 × 2 × 2 factorial experiment was designed with factors of anemone symbiont type, irradiance level, and temperature. Two experiments were run sequentially in one incubator. For each, 28 anemones, consisting of 14 zoochlorellate anemones and 14 zooxanthellate anemones, were placed in individual 50-ml beakers containing 35 ml of 5 μm-filtered seawater. For the first experiment the anemones were incubated at 20°C; for the second experiment the anemones were incubated at 13°C. The beakers containing the anemones were arranged randomly within the incubator under a bank of fluorescent lights providing a mean irradiance of 100 μmol photons/m^2/s. For each experiment, half of each group of anemones was covered with mesh for the low irradiance treatment (10% of full irradiance; see Saunders and Muller-Parker, 1997, for details). The lights were set to a natural daylength cycle of 14 h:10 h (light:dark). The anemones were fed every three days with freshly hatched *Artemia* nauplii and were last fed two days prior to ^{14}C incubation. The anemones were maintained under the experimental conditions for 21 days prior to measuring carbon fixation and translocation rates.

Carbon fixation and translocation

The amount of carbon photosynthetically fixed by the algal symbionts and translocated to the anemone host was measured using the ^{14}C method (O'Brien, 1980; Battey and Patton, 1987), with some modifications. One hour prior to the ^{14}C incubation period each anemone was transferred to an individual clear plastic vial (Nunc® tube). Exactly 10 ml

of 5 μm-filtered seawater was added to each vial and the anemones were returned to their treatment conditions.

The ^{14}C incubations were always begun at the same time of day (0900 h) to minimize variation due to any factors associated with the natural photoperiod of the anemone. The addition of ^{14}C-bicarbonate to each vial was noted as time zero. After thorough mixing, 100 μl of the seawater was subsampled to determine the total activity of the seawater in the vial, which ranged from 13.6 to 21.3 μCi/anemone. Anemones in vials that were covered completely with foil to exclude light served as controls for each experiment. These controls were used to account for dark fixation of ^{14}C by the algae and/or the animal under each set of conditions. Separate controls were run for zoochlorellate and for zooxanthellate anemones. All anemones were incubated with ^{14}C for 1.5 h under the appropriate temperature and irradiance conditions they had experienced for 21 days. After incubation, the anemones were rinsed thoroughly with non-labelled seawater, making sure that seawater retained in the coelenteron was also expelled. The seawater in the vials was replaced, and all of the vials were covered completely with foil. The vials were then returned to the appropriate incubation conditions for the dark chase period, which was 1.75 h for most experiments. Following the dark chase period, the anemones were rinsed again and individually homogenized in seawater with a motor-driven teflon tissue grinder (60 ml volume). Homogenate volume (= anemone) was measured and 1 ml of the homogenate was frozen for later protein analysis. A 0.5 ml sample of the homogenate was transferred to a 7-ml plastic scintillation vial and acidified with 0.3 ml 6 *N* HCl under a heat lamp in a fume hood to remove unincorporated inorganic ^{14}C label. Assay of homogenate was used to determine the amount of ^{14}C fixed by the whole anemone.

The algae were separated from the host fraction to measure the distribution of ^{14}C in both fractions. Ten ml of the homogenate was centrifuged in a table top swinging bucket centrifuge for 10 min. The algal pellet was rinsed two times and the final algal pellet was resuspended in 5 ml of filtered seawater. The combined supernatant was the animal fraction of the homogenate and the resuspended pellet was the algal fraction. The final animal fraction volume was measured and 1-ml samples of the animal and algal fraction were frozen for later analysis. Half-milliliter (0.5-ml) samples of each fraction were acidified with 0.3 ml 6 *N* HCl, as described above. The acidified homogenate, animal, and algal samples in the scintillation vials were then neutralized with 0.3 ml 6 *N* NaOH, 5 ml of Ecolume scintillation fluid was added, and disintegrations per minute (DPM) of each sample counted in a Packard TriCarb 1900TR liquid scintillation counter.

To compare translocation of ^{14}C by freshly collected field anemones to the anemones in the experimental treatments, anemones gathered from the field were subjected to ^{14}C analysis the day after collection. These anemones were kept under a light bank of fluorescent lamps at a photosynthetically saturating irradiance of 309 μmol photons/m^2/s in a flow-through ambient seawater table (11°C) until ^{14}C analysis.

Biomass parameters

The protein content of the homogenate and animal fractions of each anemone was determined by the method of Lowry (Lowry *et al.*, 1951), using bovine serum albumin (BSA) as a standard. Two replicates of both homogenate and animal fractions from each anemone were analyzed on a Hitachi 100-40 spectrophotometer. To ascertain the algal biomass and proportion of zoochlorellae and zooxanthellae in each anemone, cell counts were done on the frozen algal fractions. The number of each alga (zoochlorellae and zooxanthellae) in each sample was counted using a hemacytometer viewed under a compound microscope. Six replicate counts of algal numbers were done for each sample. The mean of the replicate counts was normalized to weight of anemone homogenate protein to provide an estimate of algal density in each anemone.

Percent carbon translocation

The percent of fixed ^{14}C translocated to the host during the 1.75-h dark chase time was determined by dividing the DPM calculated for the whole animal fraction by DPM in the whole homogenate fraction. Any dark carbon fixation by the algae and host was accounted for by subtracting the mean DPM per mg protein of the dark control fractions for the appropriate symbiont type from the DPM per mg protein of each experimental anemone fraction (homogenate or animal) before calculating the percent translocation. For all symbiotic anemones, dark fixation accounted for less than 10% of the total carbon fixed by anemones in the light. For the nonsymbiotic anemones, dark fixation accounted for 86% of the total carbon fixed. Because the data were in the form of percentages, they were arcsine transformed for statistical analysis.

Rates of carbon fixation and translocation

Although the percent of fixed carbon translocated to the host is important, it does not indicate the actual rate of carbon received by the anemone under different environmental conditions. For that information, the rates of carbon fixation and translocation must be examined. The specific activity of ^{14}C in the seawater was used to calculate the actual amount of carbon fixed and translocated. The weight of carbon dioxide (all forms) present in the seawater was determined by the alkalinity method described in Parsons *et al.* (1984). The weight of the total inorganic carbon present in the seawater was then multiplied by the rate of uptake (or

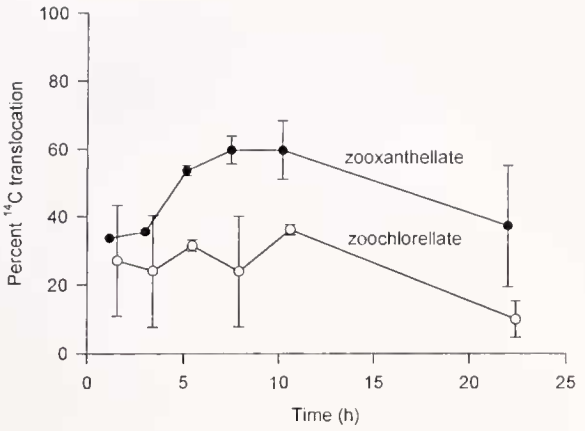

Figure 1. The effects of symbiont type and dark chase period on the percent of carbon translocated to the host anemone. $n = 2$ for each group; ± 1 SD of the mean.

translocation) of the labelled carbon in the sample, as determined by dividing DPM in the homogenate (or animal) fraction sample (corrected for DPM in the dark control) by the total activity (DPM) of the ^{14}C added and the hours of incubation with ^{14}C. The result is the rate of carbon fixation (or translocation), as amount of C fixed (or translocated) per hour.

Carbon fixation and translocation rates can be expressed on the basis of both anemone biomass (protein) and on the basis of an individual algal cell. Comparison of rates normalized to these two parameters shows how algal density affects photosynthesis and translocation. The rate of carbon fixed by anemones was calculated by using the homogenate fractions in the above calculation and normalizing to either anemone protein biomass or to number of algae. The rate of carbon translocated to the animal was calculated by using the animal fractions in the above calculation.

All analyses of variance and multiple range test statistics were examined with a significance level of 5%. Statistics were calculated using Statistix 4.1 by Analytical Software.

Results

Percent C translocation over time

A ^{14}C pulse-chase time course experiment was conducted with field anemones to determine if and how the length of the dark chase time affected the percent of carbon translocated to the host by the two symbionts. A 2 × 6 factorial analysis of variance showed that symbiont type had a significant effect on percent translocation ($P < 0.000$). Over the entire chase time period, the percent of fixed carbon translocated to the host by zooxanthellae is significantly higher than the percent of fixed carbon translocated by zoochlorellae (Fig. 1). The length of the chase time period also significantly affected the percent of carbon translocated to the host anemone ($P = 0.031$), but there was no interaction between symbiont type and chase period. Tukey's (HSD) multiple range test indicated that only chase time periods of 10.2 h and 22 h are significantly different from each other. To permit direct comparison of the effects of external factors (temperature and irradiance) on percent translocation, we used a short dark chase period (1.75 h) to compare C translocation of zoochlorellae and zooxanthellae in all subsequent experiments.

Percent translocation

There was no significant difference in the percent of carbon translocated from the algae to the animal in zoochlorellate, zooxanthellate, and mixed anemones collected from the field and incubated under saturating irradiance and at ambient seawater temperature (comparison by ANOVA). Percent carbon translocated averaged 30% for all field anemones under these conditions (Fig. 2).

The percent C translocated was higher for anemones maintained under the experimental treatments than for field anemones, and zoochlorellae translocated a greater percent of carbon (up to 65%; Fig. 2). Both temperature and symbiont type are significant main effects on percent translocation. Both symbionts translocated greater percentages of fixed carbon at 20°C than at 13°C (2 × 2 × 2 factorial analysis, $P = 0.013$). Additionally, zoochlorellae translocated a higher percent of fixed carbon than zooxanthellae ($P = 0.036$) at both temperatures. Irradiance was not a significant main effect on the percent of carbon translocated to the host ($P = 0.437$). No interaction effects were significant. Although these results show that hosting zoochlorel-

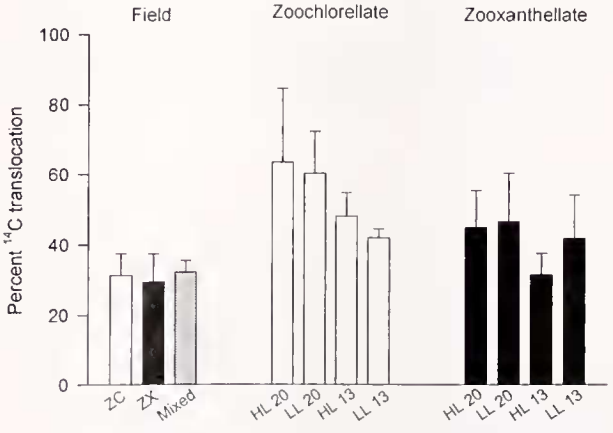

Figure 2. Percent of carbon translocated to the anemone host after a 1.75 h dark chase period. Field anemones were incubated at 11°C and a light intensity of 309 μmol photons/m^2/s (for zoochlorellate anemones, $n = 4$; for zooxanthellate and mixed anemones, $n = 2$). Experimental zoochlorellate and zooxanthellate anemones were incubated under their treatment conditions: high light (HL, 100 μmol/m^2/s) or low light (LL, 10 μmol/m^2/s) at either 13 or 20°C (20 or 13). $n = 5$ for each group; ± 1 SD of the mean.

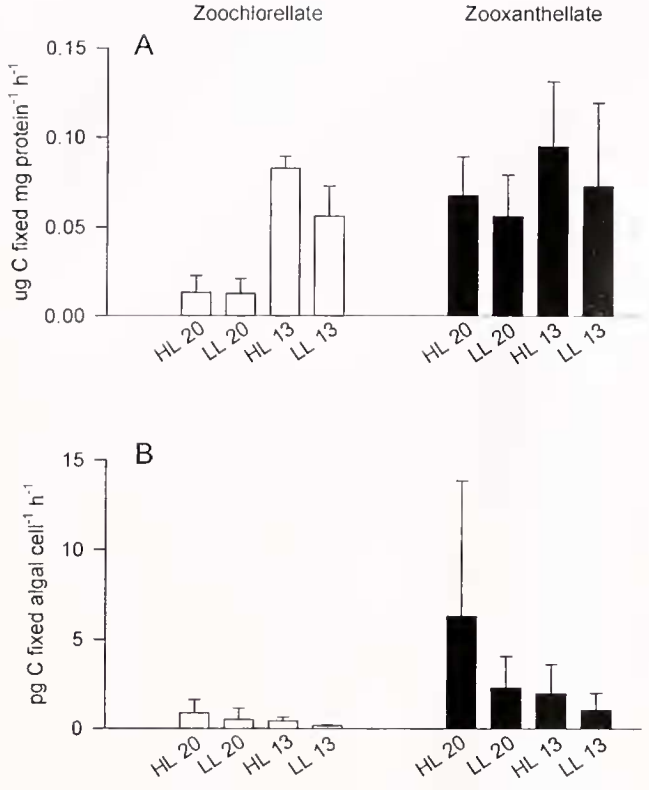

Figure 3. The rate of carbon fixation by zoochlorellate (□) and zooxanthellate (■) anemones incubated under their treatment conditions: high light (HL, 100 μmol/m^2/s) or low light (LL, 10 μmol/m^2/s) at either 13 or 20°C (20 or 13). $n = 5$ for each group; ± 1 SD of the mean. A. The rate of carbon fixation per mg anemone protein. B. The rate of carbon fixation per algal cell.

lae at higher temperatures results in a greater *percent* of fixed carbon to the anemone, carbon translocation rates are needed to compare the actual *amounts* of carbon received by zoochlorellate and zooxanthellate anemones under field and experimental conditions.

Rates of carbon fixation and translocation

The rate of carbon fixation by zoochlorellate and zooxanthellate anemones maintained under high and low irradiance at 13°C and 20°C for 21 days was significantly affected by an interaction between temperature and symbiont type ($P = 0.009$). While zooxanthellate anemones fixed carbon at the same rate at both temperatures, zoochlorellate anemones fixed about three times more carbon at 13°C than at 20°C for rates expressed on the basis of anemone biomass (Fig. 3a). Carbon fixation and translocation rates expressed on an algal cell basis are needed to compare these processes at the level of the individual algal cell with that of the symbiotic association. When the rate of carbon fixation is normalized to algal numbers instead of to anemone protein biomass, none of the interaction effects were significant and both algae fixed carbon at a *lower* rate at 13°C than at 20°C (2.3 times less and 3 times less, respectively; $P = 0.004$; Fig. 3b). The rate of carbon fixation per algal cell is significantly greater under high irradiance than under low irradiance ($P = 0.045$), and at both temperatures the zooxanthellae fixed carbon at a significantly greater rate than did the zoochlorellae ($P = 0.000$).

As shown in Figure 4a for carbon fixation rates normalized to anemone biomass, the rate of carbon translocated to the host anemone is significantly affected by an interaction between temperature and symbiont type ($P = 0.009$). While zooxanthellate anemones experienced similar rates of carbon translocation at both temperatures, rates of translocation in zoochlorellate anemones were almost 3.5 times less at 20°C than at 13°C (Fig. 4a). At 13°C, rates of translocation are comparable for both zoochlorellate and zooxanthellate anemones, and these rates were higher at the high irradiance level at both temperatures (Fig. 4a). When carbon translocation rates are normalized to algal cell number, a significant interaction between temperature and symbiont type is again observed ($P = 0.039$; Fig. 4b). In this case, the rate of carbon translocation was also greater per

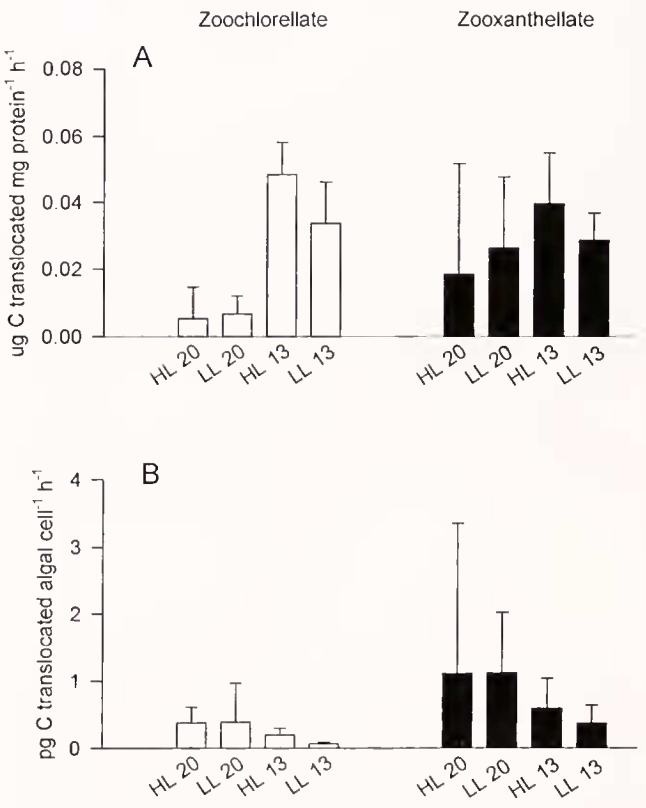

Figure 4. The rate of carbon translocation by zoochlorellate (□) and zooxanthellate (■) anemones incubated under their treatment conditions: high light (HL, 100 μmol/m^2/s) or low light (LL, 10 μmol/m^2/s) at either 13 or 20°C (20 or 13). $n = 5$ for each group; ± 1 SD of the mean. A. The rate of carbon translocation per mg anemone protein. B. The rate of carbon translocation per algal cell.

Table I

Rates of carbon fixation and translocation by algae in zoochlorellate, zooxanthellate and mixed field anemones collected during summer, normalized to anemone protein biomass or to alga

	CARBON FIXED		CARBON TRANSLOCATED	
ANEMONE TYPE	μg C fixed/mg protein/h	pg C fixed/ alga/h	μg C translocated/mg protein/h	pg C translocated/ alga/h
Zoochlorellate	0.110 ± 0.03	0.275 ± 0.14	0.034 ± 0.007[a]	0.091 ± .06
Zooxanthellate	0.145 ± 0.06	1.236 ± 1.13	0.038 ± 0.004[a]	0.390 ± 0.042
Mixed	0.199 ± 0.02	0.684 ± 0.08	0.065 ± 0.012[b]	0.221 ± 0.01
Results of 1-way ANOVA	NS	NS	$P = 0.014$	NS

For zoochlorellate anemones, $n = 4$; for zooxanthellate and mixed anemones, $n = 2$. NS denotes the parameters (column headings) that are not significantly different among the three anemone types. Tukey's HSD Multiple Range Test indicated that both zoochlorellate and zooxanthellate anemones experienced similar rates of translocation per mg protein, while mixed anemones experienced a significantly greater rate of translocation per mg protein (a and b are used to indicate these differences among anemone types).

zooxanthella than per zoochlorella at both 13°C and 20°C; however, while zooxanthellae translocated approximately 2.5 times less carbon at 13°C as at 20°C, zoochlorellae translocated almost 4 times less carbon at 13°C as at 20°C (comparisons between temperatures use pooled rates from both irradiance levels, because irradiance did not affect the rate of carbon translocation per algal cell).

Although our sample size for field anemones is small, data obtained from these anemones provide a valuable comparison to treatment anemones. When mixed anemones are included in the comparison of carbon fixation and translocation rates of field anemones, the carbon fixation rates of zoochlorellate, zooxanthellate, and mixed field anemones are not significantly different from each other, whether expressed on the basis of anemone protein biomass or algal cell (Table I). Although algal cell-based translocation is not significantly different, the rate of carbon translocation per mg protein in *A. elegantissima* is significantly affected by symbiont type (Table I). However, Tukey's HSD Multiple Range Test indicated that both zoochlorellate and zooxanthellate anemones experienced similar rates of translocation per mg protein, while mixed anemones experienced a significantly greater rate of translocation per mg protein.

Algal density in anemones

Zoochlorellate field anemones contained significantly higher algal densities than did zooxanthellate field anemones (Fig. 5; $P = 0.000$). Mixed anemones had algal densities between those of zooxanthellate and zoochlorellate anemones; the density of algae in mixed anemones was not significantly different from the density of algae in either zooxanthellate or zoochlorellate anemones.

A two-way ANOVA performed on the algal density within the anemones after 21 days under the experimental treatments showed that the interaction between temperature and symbiont type was significant ($P = 0.001$). All anemones held at 20°C contained similar densities of algae; however, at 13°C zooxanthellate anemones had significantly fewer algae per mg anemone protein than did zoochlorellate anemones (Fig. 5). Anemones held in the laboratory under all experimental treatments contained significantly fewer algae than did anemones freshly collected from the field ($P = 0.000$).

Discussion

Percent translocation and translocation rates

In the field, zoochlorellate and zooxanthellate anemones receive the same amount of photosynthetic carbon from their symbionts during the summer in northern Puget Sound (Fig. 2, Table I). These results suggest that during summer

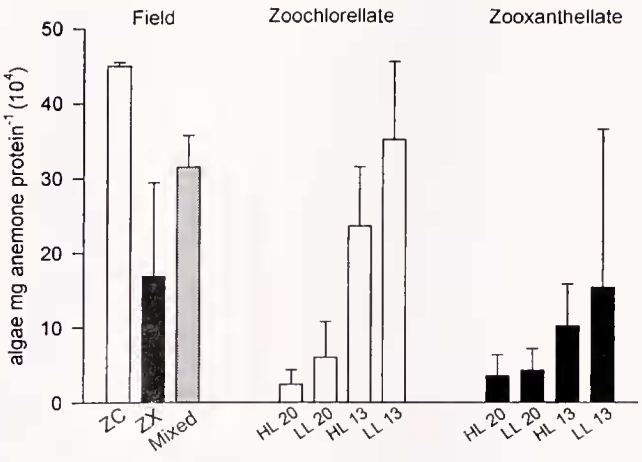

Figure 5. Density of algae in field anemones ($n = 20$, 17, and 3 for zoochlorellate, zooxanthellate, and mixed anemones respectively) and in zoochlorellate (□) and zooxanthellate (■) anemones after 21 days under high light (HL, 100 μmol/m^2/s) or low light (LL, 10 μmol/m^2/s) at either 13 or 20°C (20 or 13). $n = 7$ for each group; ± 1 SD of the mean.

there is no selective advantage, with respect to carbon, of hosting one symbiont over the other under saturating irradiance levels and ambient temperature. However, under different environmental conditions imposed in a laboratory experiment, zoochlorellae translocated a greater percent of fixed carbon to the host than did zooxanthellae, and both algal symbionts translocated a significantly greater percent of the carbon they fixed at 20°C than at 13°C (Fig. 2). The implications of these results are discussed below.

In our study, zoochlorellae translocated a much greater percent of the fixed carbon than shown by the previous studies of Muscatine (1971), O'Brien (1980), and Verde and McCloskey (1996). However, the percent carbon translocated by both algae in *A. elegantissima* is comparable to values obtained for other temperate cnidarian symbioses (Sutton and Hoegh-Guldberg, 1990; Davy *et al.*, 1997). Muscatine (1971), using ^{14}C analysis, determined that zoochlorellae translocate only 1.0% to 3.6% of the carbon they fix. However, Muscatine used only the tentacles and not whole anemones in his experiments; in addition, for some experiments the animal and algal fractions from tentacles were homogenized and separated before incubation with ^{14}C. O'Brien (1980) found that zoochlorellae translocated 1.3% to 3.9% of the carbon they fixed. O'Brien also used only tentacles of *A. xanthogrammica*. He dissected the epidermis of the anemone from the algae-containing gastrodermis after ^{14}C incubation and used the epidermis as the animal fraction and the gastrodermis as the algal fraction for translocation calculations. Any labelled carbon that the algae had translocated to the gastrodermal tissues of the host was counted as fixed carbon retained by the algal fraction. In addition, any host mechanisms acting upon translocation would be lost due to the excision of the tentacle from the remainder of the anemone body.

The ^{14}C method employed in this study accounts only for short-term carbon products fixed and released by the algae from inorganic carbon supplied in the external environment. There is substantial evidence for zooxanthellae that recently fixed carbon is released to the host (Sutton and Hoegh-Guldberg, 1990; Wang and Douglas, 1997). In contrast, translocation of carbon based on the growth-rate method takes into account the daily carbon budget of the symbiotic algae (Muscatine *et al.*, 1984). Because carbon required for algal growth may be supplied from the host animal (Trench, 1979), any contribution of host-derived carbon is wholly missed by the ^{14}C method as applied here. This may explain the discrepancy between our results and those of Verde and McCloskey (1996), who found that zoochlorellae may have only minimal excess carbon available to translocate to the host. The algae may selectively translocate photosynthetically fixed carbon while concurrently obtaining carbon for growth from the anemone host. This comparison also illustrates the importance of defining the time scales used to assess carbon translocation. Zoochlorellate and zooxanthellate anemones receive the same amount of translocated carbon during short-term (hours) ^{14}C incubations (our results), while growth rate comparisons based on longer time intervals (days to weeks) show that zoochlorellae translocate less carbon (Verde and McCloskey, 1996). The appropriate time scale for comparisons of these two algae will depend on the metabolic fate of the translocated carbon and on the external supply of carbon derived from host feeding.

Higher carbon fixation rates by both algae at the high irradiance level at both temperatures also resulted in greater carbon translocation rates (Figs. 3, 4). It appears that the symbiotic algae simply translocate fixed carbon at a higher rate under high irradiance because they have more photosynthetic product available. These results indicate that, with similar algal densities, anemones located in areas exposed to high solar irradiance should receive larger amounts of fixed carbon from their symbionts than should anemones located in areas of low light. The same is true for temperature. Both zoochlorellae and zooxanthellae fixed and translocated carbon at greater rates at 20°C. However, the advantage of greater carbon translocation at the higher temperature and irradiance level on an algal cell basis is offset by lower algal densities under these conditions, reducing the amount of carbon received by the anemone (see below).

Algal density and carbon translocation in anemones

Zoochlorellate anemones from the field contained approximately two to three times the density of algae as did zooxanthellate anemones (Fig. 5), as has been found by others (Verde and McCloskey, 1996; Dingman, 1998). Thus, although an individual zoochlorella translocates carbon to the host anemone at a lesser rate than does a zooxanthella (Table I; Fig. 4b), both anemone types receive fixed carbon at similar rates because of increased densities of zoochlorellae in field anemones (Fig. 5). Interestingly, although the zoochlorellae are numerically more abundant, volume comparisons indicate that they occupy the same "space" as the larger zooxanthellae within the anemones (unpub. data). Therefore, both anemone types in the field maintain similar ratios of algal to animal biomass and receive similar amounts of photosynthate.

Anemones in all experimental treatments contained significantly fewer algae than did field anemones, and both types of anemone had lower algal densities at the higher temperature (Fig. 5). This may be related to differences in summer field conditions and laboratory incubator conditions. Although anemones were maintained at relatively low constant irradiances in the lab (an order of magnitude lower than noon irradiance levels in the field), they probably received more light on a daily basis than field anemones because of tide-related changes in water depth and rapid light extinction due to high plankton levels in summer. Field anemones also experienced pronounced daily changes in

water temperature during periods of exposure to low tide. Changes in density of symbionts may result from differences in both algal growth rate and algal expulsion rate under the experimental treatments. Although we did not measure these parameters in our study, zooxanthellate and zoochlorellate *A. elegantissima* have higher algal expulsion rates at 20°C than at 13°C (Saunders, 1995). McCloskey *et al.* (1996) also found that algal expulsion rates increase with increasing irradiance, and concluded that algal densities in *A. elegantissima* are regulated by expulsion of excess algae. In mixed anemones, the presence of the dominant symbiont is more likely due to that alga's ability to grow at a rate that meets or exceeds the rate of expulsion by the anemone and the growth rate of the other algal species. It is likely that greater numbers of algae were lost from zoochlorellate anemones than were lost from zooxanthellate anemones at 20°C since, as noted earlier, zoochlorellate anemones from the field contain higher densities of algae than do zooxanthellate anemones.

With respect to translocation of photosynthetic carbon, the relative abundance of zooxanthellae and zoochlorellae in *A. elegantissima* determines the amount of carbon translocated within anemones. How does the advantage of greater carbon translocation at the higher temperature and irradiance level on an algal cell basis affect the amount of carbon received by anemones when these also contain lower algal densities (Fig. 5)? A zoochlorellate anemone held at 13°C under high light receives 0.048 μg C/mg protein/h from its algae (Fig. 4a). To maintain this rate of carbon translocation at 20°C, the anemone would require an algal density of only 9.6×10^4 algae/mg protein because individual zoochlorellae translocate 2.5 times more at the higher temperature. However, the density of zoochlorellae at 20°C was one-fourth (26%) of this density (Fig. 5), showing that the higher translocation rate per cell was not sufficient to compensate for the reduced density of zoochlorellae at the higher temperature. A similar calculation for a zooxanthellate anemone shows that it needs 2.97×10^4 algae/mg protein at 20°C to maintain a translocation rate equivalent to that obtained at 13°C. However, zooxanthellate anemones held at 20°C contained 3.5×10^4 algae/mg protein (Fig. 5), about 18% *more* than required to maintain the translocation rate obtained at 13°C. This slightly elevated density of zooxanthellae was not sufficient to yield any significant difference in translocation rate (Fig. 4a). Using carbon translocation at 13°C as the basis of comparison, zoochlorellate anemones lost more algae than they should have at 20°C, and zooxanthellate anemones kept more algae than they needed to at this temperature. This comparison suggests that the nutritional contribution of the algae is not important to the host anemone and there is no regulation of algal densities to maintain certain carbon translocation rates. However, the cost to the host anemone of harboring symbionts at different densities is unknown. Should reduced algal densities lower the cost of maintaining the symbionts, then simply comparing carbon translocation rates is insufficient for assessing benefit to the host.

Application to the field

The *Anthopleura elegantissima*-zooxanthella nutritional relationship has been examined by determining the percent contribution of translocated carbon to animal respiration (CZAR). Shick and Dykens (1984) indicated that CZAR was greater for low intertidal (34%) than for high intertidal anemones (18%) due to self-shading of the anemone during exposure to air, while Fitt *et al.* (1982) demonstrated that CZAR for fed anemones (13%) was less than that for starved anemones (45%). In the only study to compare CZAR of anemones harboring both symbionts, Verde and McCloskey (1996) showed that CZAR for zooxanthellate anemones was much greater than CZAR for zoochlorellate anemones. The use of CZAR as a tool of comparison hinges on the assumptions that the algae will translocate all unneeded fixed carbon, that the anemone will use all of the translocated carbon, and that the form in which the fixed carbon is translocated does not matter to the anemone. Some of these assumptions may not apply to temperate anemone symbioses.

While there may be energetic advantages to the anemone to maintaining an algal population within its tissues, these advantages may be quite limited for temperate anemones (Davy *et al.*, 1997). *Anthopleura elegantissima* may not rely on carbon supplied by zooxanthellae for growth. Tsuchida and Potts (1994) demonstrated that *A. elegantissima* clones gained or lost weight in response to whether they were fed or not, regardless of whether they were kept in the light or dark, or whether they contained zooxanthellae or were algae-free. Similar results for zooxanthellate and zoochlorellate anemones were obtained by Blevins (1991). The heterotrophic supply of carbon appears to be the primary source of nutrition for these anemones. Indirect evidence for high rates of feeding under field conditions is provided by high ammonium concentrations in anemone-dominated tidepools (Jensen and Muller-Parker, 1994). Moreover, Davy *et al.* (1996) showed that reduced photosynthetic production of zooxanthellae in temperate anemones due to cloud cover, depth, and other environmental conditions could decrease the alga's translocatable carbon to just 0.7% of that fixed. Reliance on external carbon sources will be pronounced during seasonally low irradiance during the winter months. During such times the algae may represent a liability to the host, especially because algal densities in *A. elegantissima* during the winter season are the same as densities in midsummer (Dingman, 1998). In contrast with tropical symbiotic associations (Muscatine *et al.*, 1981; 1984; Davies, 1984), temperate symbiotic cnidarians like *Anthopleura* must often depend on sources outside of their

algal complement for their respiratory carbon requirements as well as their growth needs (Davy *et al.*, 1997).

On the other hand, during warm and sunny periods, translocated photosynthate may be an important source of carbon. Clark and Jensen (1982) proposed that a period of high yield during such conditions may be sufficient for the anemone hosts to keep the symbionts year-round. Because their study of the anemone *Aiptasia pallida* showed that temperature also affects the nature of the translocated products, it will be important to compare the metabolites translocated by zoochlorellae and zooxanthellae under the range of environmental conditions experienced by anemones in the field. The nature of these metabolites, and the ability of the anemone host to use translocated compounds, may be more important than the amount of carbon translocated. Temperate symbioses exposed to pronounced seasonal variations in environmental factors are ideal systems in which to explore variation in the nutritional contribution of algal symbionts to the host and the consequences for the association.

The quantity of carbon translocated, as examined in this study, is only one factor in the symbiosis between zoochlorellae, zooxanthellae, and the anemone host in temperate regions. While this factor has justifiably received the greatest attention in tropical algal-enidarian symbioses, it is not at all clear if provision of carbon is the most important benefit of the symbiosis to temperate *A. elegantissima*. If it was, our results suggest that zooxanthellae should predominate given their translocation potential under high temperature. Other selective advantages not directly related to carbon translocation must also be considered for this dual symbiosis. For example, there may be different energetic costs to hosting zooxanthellae and zoochlorellae associated with photooxidative stress resulting from photosynthesis, since host anemones must protect against toxic effects of reactive oxygen species (Shick, 1991). It would be interesting to compare antioxidant defenses in zooxanthellate and zoochlorellate anemones. There may be behavioral costs associated with harboring these two algae. If photosynthesis of zooxanthellae and zoochlorellae results in different expansion and contraction behaviors of anemones in the field, these may affect primary productivity and feeding on zooplankton (Shick and Dykens, 1984), as well as gas and dissolved organic matter exchanges with the environment. Ecological consequences of harboring different symbionts must also be considered. For example, Augustine and Muller-Parker (1998) have shown that selective predation on zooxanthellate anemones by a sculpin favors the survival and propagation of zoochlorellate anemones. Future studies should also focus on long-term comparisons of the growth and asexual reproduction of zooxanthellate and zoochlorellate anemones under a variety of environmental conditions. Continuing studies of this dual symbiosis in a temperate environment should prove useful to researchers studying tropical symbioses as well.

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